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(54) **Recombinant DNA, bacterium of the genus pseudomonas containing it, and process for preparing lipase by using it.**

(57) A novel recombinant DNA, capable of being replicated in a bacterium of the genus Pseudomonas, and in which a DNA containing a gene that cords for lipase has been inserted into a wide host range plasmid vector.

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Recombinant DNA, Bacterium of the Genus Pseudomonas Containing It, and Process for Preparing Lipase by Using It

The present invention relates to a recombinant DNA, a bacterium of the genus Pseudomonas containing it, and a process for preparing lipase by using it.

Lipase is an enzyme capable of hydrolyzing a lipid, and utilized in an extensive field such as application in fats and oils processing, diagnostic reagents, detargents, etc.

Hitherto, there are reports on the preparation of the lipase producing bacteria of the genus Pseudomonas by utilization of recombinant DNA techniques (Ohtera et al's Japanese Patent Laid-Open No. 60-188072, Kugimiya et al's Japanese Patent Laid-Open No. 62-228279).

These, however, are techniques in which the lipase is produced using Escherichia coli as a host.

As well known, in the instance where Escherichia coli is used as a host, products are accumulated in bacterial bodies, and little secreted into culture mediums, so that the Escherichia coli must be crushed by ultrasonic treatment etc. followed by collection of the products, bringing about disadvantages in the production of the substances such as enzymes.

Now, the present inventors considered that it may be possible to accumulate lipase in a large quantity in the culture medium if DNA fractions containing lipase genes, and plasmids are used, and also transformants comprised of bacteria of the genus Pseudomonas as a host are obtained, and they made intensive studies to accomplish the present invention.

The present invention provides a recombinant DNA, a bacterium of the genus Pseudomonas containing it, and a process for preparing lipase by using it, which process comprises separating a DNA fraction originating from the bacterium of the genus Pseudomonas and containing a lipase gene to obtain plasmids having said DNA fraction, inserting DNA into the bacterium of the genus Pseudomonas by use of these plasmids to obtain a transformant, culturing said transformant to accumulate lipase outside the bacterial body in a large quantity, and collecting the accumulated lipase.

According to one aspect of the present invention, there is provided a novel recombinant DNA, capable of being replicated in a bacterium of the genus Pseudomonas, and in which a DNA containing a gene that cords for lipase has been inserted into a wide host range plasmid vector.

According to another aspect of the present invention, there is provided a novel bacterium of the genus Pseudomonas, inserted with a novel recombinant DNA in which a DNA that originates from a bacterium of the genus Pseudomonas and carries genetic information of the lipase has been inserted into a vector.

According to still another aspect of the present invention, there is provided a novel DNA, in which a DNA that originates from a bacterium of the genus Pseudomonas and carries genetic information of the lipase comprises a structural gene of the lipase and a gene participating in the production of the lipase.

According to a further aspect of the present invention, there is provided a novel DNA, in which a structural gene of the lipase and a gene participating in the production of the lipase comprise the amino acid sequences as shown in Figs. 3A and 3B, respectively.

According to an additional aspect of the present invention, there is provided a process for producing lipase, comprising;
inserting into a host bacterium a novel recombinant DNA in which a DNA that originates from Pseudomonas and carries genetic information of the lipase has been inserted into a vector;
culturing said bacterium to cause the lipase to be produced in a cultured product; and
collecting the lipase from said cultured product.

Fig. 1 shows as an example a restriction map prepared by separating by various restriction enzymes a plasmid pLiPI having the DNA arrangement that codes for lipase of Pseudomonas cepacia M-12-33 (FERM BP-2293) and on the basis of the measurement by use of an agarose gel electrophorasis.

Fig. 2 shows a result of examination on whether or not a halo is formed in a triolein culture medium in respect of a product resulted from transformation of a Pseudomonas cepacia HW10 strain by subcloning to pFL100, of each DNA fraction obtained by cleavage of said plasmid pLiPI with various restriction enzymes.

Figs. 3A and 3B each show the amino acid sequence of the DNA that carries the genetic information of the lipase of Pseudomonas cepacia, in which Fig. 3A shows a structural gene of lipase and Fig. 3B shows an amino acid sequence of the DNA that participates in the occurrence of lipase. The underlined portion in Fig. 3A indicates the region at which the N-terminal amino acid sequence of a purified lipase protein has been determined.

A source of the chromosomal DNA containing the lipase gene of the present invention is supplied from

bacteria of the genus Pseudomonas having the ability of producing lipase. For example, it includes the strain named Pseudomonas sp. M-12-33 isolated by Arima et al [Agr. Biol. Chem., 30, 515 (1966)] . This strain has the following bacteriological properties.

(a) Morphology:

(1) Rods (0.5 to 1.0 x 1.2 to 3.0 μ).
(2) Singly or short chains
(3) Motile by one to three polar flagella.
(4) No spore.
(5) Gram stain: Negative.
(6) Acid-fast: Negative.
(7) Polymorphism: None.

(b) Growth state:

(1) Nutrient agar plate culture: Circular, convex, smooth and thin on its surface, and yellowish white and transparent.
(2) Nutrient slant culture: Filamentous, smooth on peripheries with moderate growth, convex, and pale yellowish white wigh slight gloss.
(3) Nutrient liquid culture: Moderate growth, forming a thin film on the surface, and turbid.
(4) Nutrient gelatin stab culture: Ordinary growth, and liquefied in a funnel form.
(5) Litmus milk: Slightly alkaline, reducing litmus, and liquefied, but slightly forming a precipitate.

(c) Growability:

(1) MacConkey medium: Growth.
(2) KCN medium: No growth.
(3) SS agar medium: Growth.

(d) Physiological properties:

(1) Reduction of nitrate: Positive (succinic acid, sodium nitrate medium)
(2) Denitrification reaction: Negative.
(3) MR test: Negative.
(4) VP test: Negative.
(5) Formation of indole: Negative.
(6) Formation of hydrogen sulfide: Negative.
(7) Hydrolysis of starch: Negative.
(8) Utilization of citric acid: Positive (Simmon's citrate medium).
(9) Utilization of inorganic nitrogen source: Nitrate is not utilized, but ammonium salt is utilized.
(10) Formation of pigment:
King A medium: Pale-yellow pigment.
King B medium: Pale-brown water-soluble pigment.
S.C.D. medium: Pale-brown pigment.
Tyrosine medium: Slightly-brown water-soluble pigment.
Glutamic acid agar medium: Pale-yellow water-soluble pigment.
(11) Tween 80 decomposition: Positive.
(12) Casein decomposition: Positive.
(13) PHB accumulation: Positive.
(14) Urease: Negative.
(15) Oxidase: Positive.
(16) Catalase: Positive.
(17) Arginine dihydrolase: Negative.

(18) Lysine dicarboxylase: Positive.
(19) Ornithine dicarboxylase: Positive.
(20) Acyl amidase: Positive.
(21) Growth pH: 5.0 to 9.0
(22) Growth temperature:
10 °C (+), 20 °C (+ +), 25 °C (+ + + +), 30 °C (+ + + +), 35 °C (+ +), 37 °C (-), 42 °C (-).
(23) Behavior to oxygen: Aerobic.
(24) O-F test: Oxidative.
(25) Formation of acid and gas from saccharides:
a) Formation of acid: As shown in Table 1.
b) Formation of gas: Negative.

Table 1

Saccharides	Formation of acid
L-arabinose	+
D-xylose	+
D-glucose	+
D-mannose	+
D-fructose	+
Maltose	+
Sucrose	+
Lactose	+
Trehalose	+
D-sorbitol	+
D-mannitol	+
Inositol	+
Glycerol	+
Starch	-
D-galactose	+

The above taxonomical properties were studied with reference to Manual of Microbiological Method (edited by American Bacteriological Society). SAIKINGAKU JISSHU TEIYOU (Manual of Bacteriology Practice); edited by The University of Tokyo, Friend Society of Infections Diseases Research Institute), etc., and comparison of these properties with what are described in Bergey's Manual of Determinative Bacteriology 8th Edition, Robert E. et al's Gramnegative Organisms: An approach to Identification (Guide to Presumptive Identification) and Cowan's Manual for the Identification of Medical Bacteria, revealed that almost all the properties except the growth temperature are coincident with those of Pseudomonas cepacia. Thus, the present bacterium was named Pseudomonas cepacia M-12-33. This strain is deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, under FERM BP-2293.

Preparation of transformant

The chromosomal DNA and transformant according to the present invention can be prepared by the following procedures.

(1) Procedure for the preparation of chromosomal DNA:

The Pseudomonas cepacia M-12-33 strain (FERM BP-2293) is cultured overnight at 30 °C under aerobic conditions, using an LB medium (tryptone: 1.0 %; yeast extract: 0.5 %; sodium chloride: 1.0 %). Bacterial bodies are collected, followed by extraction and purification of the chromosomal DNA by utilizing a

known method such as the Saito and Miura's method [Biochem. Biophys. Acta., 72, 619-629 (1963)] .

(2) Procedure for the insertion of DNA fractions into plasmid vectors and the transformation

Plasmid vectors may preferably be vectors capable of being replicated in a host, having a known restriction enzyme incision portion, and having a selective marker for chemical resistance and the like. For example, they include wide host range plasmid vectors such as RSF1010, R1b679 and R1162. More specifically, there can be used pFL100 obtained by incorporating a kanamycin-resistant gene into RSF1010.

The vector DNA thus obtained is cleaved with a restriction enzyme such as EcoRI, to which the chromosomal DNA is cleaved with the like restriction enzyme and purified is joined according to a known method using ligase or the like, thus obtaining a recombinant plasmid.

Subsequently, using the recombinant plasmid, bacteria of the genus Pseudomonas as exemplified by Pseudomonas cepacia, Pseudomonas putida, etc. are subjected to transformation.

The transformation is carried out by utilizing a calcium chloride method, a rubidium chloride method, an electroporation method.

Transformed bacterium having the ability of producing lipase may be selectively separated with simplicity by using an agar medium containing triglycerides such as tributyrin and triolein emulsified with polyvinyl alcohol, and having an antibiotic substance in a predetermined concentration.

Namely, this is because the bacteria containing lipase genes can be selectively separated by separating from among the transformed bacteria a strain that forms a large clearing zone in the agar medium.

From the resulting bacteria, a plasmid DNA containing the lipase gene is obtained by using a known method such as an alkali method or a boiling method.

The lipase is further produced by using the bacteria of the genus Pseudomonas obtained by insertion of the thus obtained plasmid DNA according to the present invention. For example, the Pseudomonas cepacia M-12-33 strain having the novel recombinant plasmid is cultured by using a known medium, and the lipase can be obtained by separating it from the culture supernatant and purifying the separated one.

Method for measuring lipase activity

In a test tube with a flat bottom, 5 mℓ of a substrate solution emulsified by mixing 75 mℓ of olibe oil, in 225 mℓ of 2 % polyvinyl alcohol (PVA) solution, and 4 mℓ of a 0.2 M McIlvaine's buffer solution (pH 7) are charge and the mixture is pre-warmed at 37° C for 5 minutes. To this mixture, 1 mℓ of a specimen solution is added and thoroughly mixed with shaking, and immediately the resulting solution is left to stand at 37° C for 30 minutes. After 30 minutes, 10 mℓ of an aceton/ethanol (1:1) mixed solution is added and thoroughly mixed with shaking. To this solution, 10 mℓ of a 0.05 N sodium hydroxide solution and 10 mℓ of an acetone/ethanol (1:1) mixed solution are added, followed by further addition of 2 drops of a phenolphthalein reagent to carry out titration up to pH 10.00 using 0.05 N hydrochloric acid, while blowing nitrogen gas on the surface of the solution with stirring using a stirrer. The blank determination is similarly operated using purified water in place of the specimen solution. The enzyme titer is assumed to be 1 unit when 1 micromol of a fatty acid is formed in 1 minute.

The present invention will be specifically described below by giving Example.

Example 1

(1) Preparation of chromosomal DNA:

Pseudomonas cepacia M-12-33 (FERM BP-2293) was cultured overnight at 30° C under aerobic conditions with shaking by use of the LB medium, and, after collection of bacteria, the chromosomal DNA was extracted and purified according to a DNA extraction method by the Saito and Miura's method, thus obtaining 8.4 mg of the chromosomal DNA.

(2) Insertion of DNA fractions into plasmid vectors:

A wide host range plasmid vector RSF1010 (330 ng) was cleaved with a restriction enzyme PstI to extract an 8.1 Kb fraction. On the other hand, a 1.4 Kb fraction containing a kanamycin resistant gene was prepared from 480 ng of pUC-4K (available from Pharmacia Fine Chemicals, Inc.) according to the similar operation. Both were joined with a T4 DNA ligase, thereby obtaining a plasmid vector pFL100. The pFL100 was purified from an Escherichia coil C600 strain according to Maniatis et al's method [Maniatis et al. Molecular Cloning, a laboratory manual, 92 - 94 (1981)].

The above chromosomal DNA (8.4 µg) was taken up, and, with addition of a restriction enzyme EcoRI, reacted at 37 °C for 15 minutes, to cleave the DNA in part. On the other hand, the restriction enzyme EcoRI was added to 2.5 µg of the plasmid pFL100 to carry out the reaction at 37 °C for 2 hours, thus completely cleaving the DNA. To the cleaved plasmid DNA, alkaline phosphatase was added to effect dephosphorylation. The cleaved chromosomal DNA and the vector plasmid DNA were mixed, and reacted with addition of DNA ligase to carry out the reaction to join DNA fractions.

(3) Transformation by plasmid:

In selecting and separating the lipase gene in the above culture medium containing triolein, a halo-formation defective host is advantageous. Accordingly, the Pseudomonas cepacia M-12-33 strain (FERM P No. 9871) was treated with nitrosoguanidine, and a variant strain HW10 that forms no halo was obtained using Antibiotic Medium 3 (available from Difco Co.) containing 0.2 % of triolein.

The present inventors found that the electroporation method is very effective for the transformation of said strain. More specifically, said strain was cultured at 30 °C using 20 mℓ of the LB medium until it entered the logarithmic growth phase (OD_{660} = 0.4), and then cooled, followed by centrifugal separation at 3,500 rpm for 5 minutes. The bacterial bodies were washed with 10 mℓ of a buffer solution (pH 7.4) comprised of 272 mM sucrose and 7 mM sodium phosphate. Centrifugal separation was again carried out, followed by suspension in 0.8 mℓ of the like buffer solution. The joined recombinant DNA was added, and a pulse of 6,000 V/cm was applied, followed by addition of 5.7 mℓ of the LB medium to carry out culture at 30 °C for 2 hours with shaking. As a result of these procedures, there was obtained a transformant strain of 2 x 10^6 cfu/ µg of DNA.

The recombinant DNA thus joined was subjected to the electroporation method to effect the transformation of the HW10 strain, and then the transformants were spreaded onto a selective medium containing 500 µg/m ℓ of kanamycin and 0.2 % of triolein. As a result, 8 halo-forming strains were obtained.

(4) Identification of transformant containing lipase gene:

Plasmids were extracted from the above 8 halo-forming strains, and cleaved with the restriction enzyme EcoRI. The inserted DNA fractions were analyzed by agarose electrophoresis to find that the strains were divided into those into which about 10 Kb of the EcoRI fraction was inserted and those containing 6.0 Kb and 2.7 Kb of EcoRI fractions. An experiment using an antilipase antiserum revealed that the desired lipase gene was present on the 10 Kb EcoRI fraction. It is considered that in fact the halo-formation defective strain HW10 did not undergo the variation on the lipase gene, but turned to the halo-formation defective strain because of the variation of other genes. Regarding the EcoRI fractions of 6.0 Kb and 2.7 Kb, it is considered that the transformant strain thereof formed halo because the gene having undergone this variation was cloned. The plasmic vector containing 10 Kb of the EcoRI fraction was named pLiPI, and its restriction enzyme cleavage map is shown in Fig. 1.

(Analysis of lipase gene)

The pLiPI plasmid was cleaved with various restriction enzymes, where each DNA fraction was subcloned to pFL100 to effect transformation of the Pseudomonas cepacia HW10 strain, and whether or not the halo were formed in the triolein medium was examined in the same manner as the above. Results obtained are shown in Fig. 2. The plasmic pLip10 obtained by the subcloning of a 3 Kb ClaI-EcoRI fraction also produced lipase. However, no production of lipase was observed as to the plasmid containing a 2 Kb ClaI-KpnI fraction.

The size of the main body of lipase gene, presumable from the fact that the lipase has a molecular weight of 34,000, is considered to be about 1.1 Kb, but the result of the subcloning shows that 2 Kb or more of the DNA fraction is required for the production of the lipase. Then, the whole base sequence of the 3 Kb ClaI-EcoRI fraction was determined according to the Mizusawa et al's method [Nucleic Acids Res., 14-(3), 1319-1324 (1986)].

As a result, it became clear that the 3 Kb ClaI-EcoRI fraction comprises a structural gene of lipase and a gene of the protein essential to the production of lipase. The amino acid sequence of these genes is shown in Figs. 3A and 3B.

(5) Production of lipase:

The Pseudomonas cepacia M-12-33 was transformed with the plasmid pLiP10 containing the above lipase gene. The present strain was cultured with shaking at 30 °C for 3 days, using a liquid medium comprising 2.0 % of soybean oil, 0.5 % of peptone (available from DIFCO Co.), 0.3 % of meat extract (available from Difco Co.), 0.1 % of KH_2PO_4, 0.02 % of $MgSO_4 \cdot 7H_2O$, 0.001 % of $FeSO_4 \cdot 7H_2O$, and one drop of Adecanol (available from Asahi Denka). A culture supernatant with a lipase activity of 600 U/mℓ was obtained by centrifugal separation. This productivity was about 40 times the lipase productivity of the host bacterium Pseudomonas capacia M-12-33 strain.

Claims

1. A novel recombinant DNA, capable of being replicated in a bacterium of the genus Pseudomonas, and in which a DNA containing a gene that cords for lipase has been inserted into a wide host range plasmid vector.

2. The novel recombinant DNA according to Claim 1, wherein said DNA containing a gene that cords for lipase is a DNA originating from a bacterium of the genus Pseudomonas.

3. The novel recombinant DNA according to Claim 1, wherein the vector DNA is plasmid RSF1010 or a plasmid originating therefrom.

4. The novel recombinant DNA according to Claim 1, wherein said plasmid originating from RSF1010 is plasmid pFL100.

5. A novel bacterium of the genus Pseudomonas, inserted with a novel recombinant DNA in which a DNA that originates from a bacterium of the genus Pseudomonas and carries genetic information of the lipase has been inserted into a vector.

6. A novel DNA, in which a DNA that originates from a bacterium of the genus Pseudomonas and carries genetic information of the lipase comprises a structural gene of the lipase and a gene participating in the production of the lipase.

7. A novel DNA, in which a structural gene of the lipase and a gene participating in the production of the lipase comprise the amino acid sequences as shown in Figs. 3A and 3B, respectively.

8. The novel recombinant microorganism according to Claim 5, wherein the novel bacterium of the genus Pseudomonas comprises Pseudomonas cepacia or Pseudomonas putida as a host.

9. The novel Pseudomonas cepacia (pLiPI) according to Claim 5, wherein the insertion of the novel recombinant DNA is carried out according to an electroporation method.

10. A process for producing lipase, comprising:
inserting into a host bacterium a novel recombinant DNA in which a DNA that originates from Pseudomonas and carries genetic information of the lipase has been inserted into a vector;
culturing said bacterium to cause the lipase to be produced in a cultured product; and
collecting the lipase from said cultured product.

11. The process for producing lipase according to Claim 10, wherein said host bacterium into which the recombinant DNA is inserted is either Pseudomonas cepacia or Pseudomonas putida.

FIG. 1

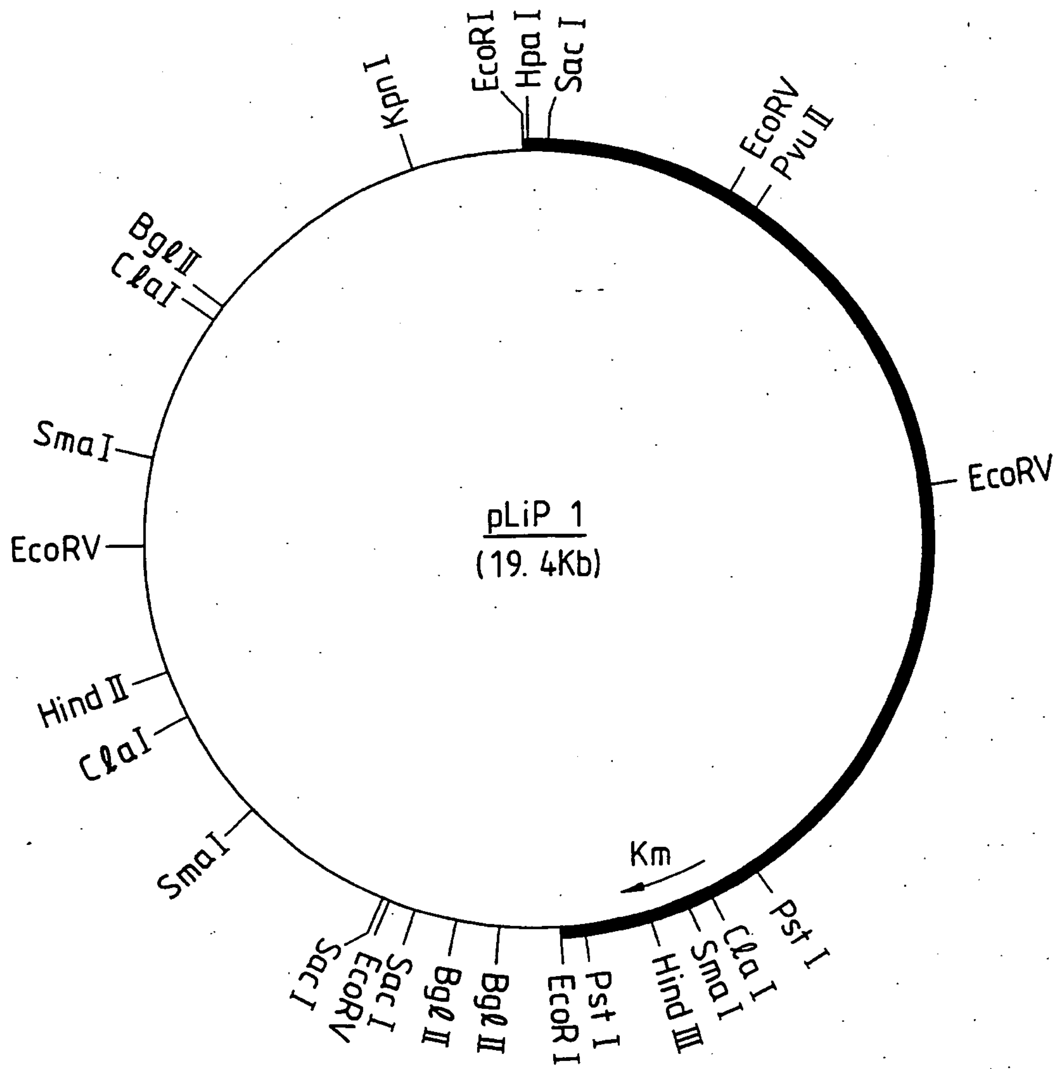
EcoRI
Hpa I
Sac I
Kpn I
EcoRV
Pvu II
Bgl II
Cla I
Sma I
EcoRV
EcoRV
Hind II
Cla I
Sma I
Km
Pst I
Cla I
Sma I
Hind III
Pst I
EcoR I
Bgl II
Bgl II
Sac I
EcoRV
Sac I
pLiP 1
(19. 4Kb)

FIG. 2

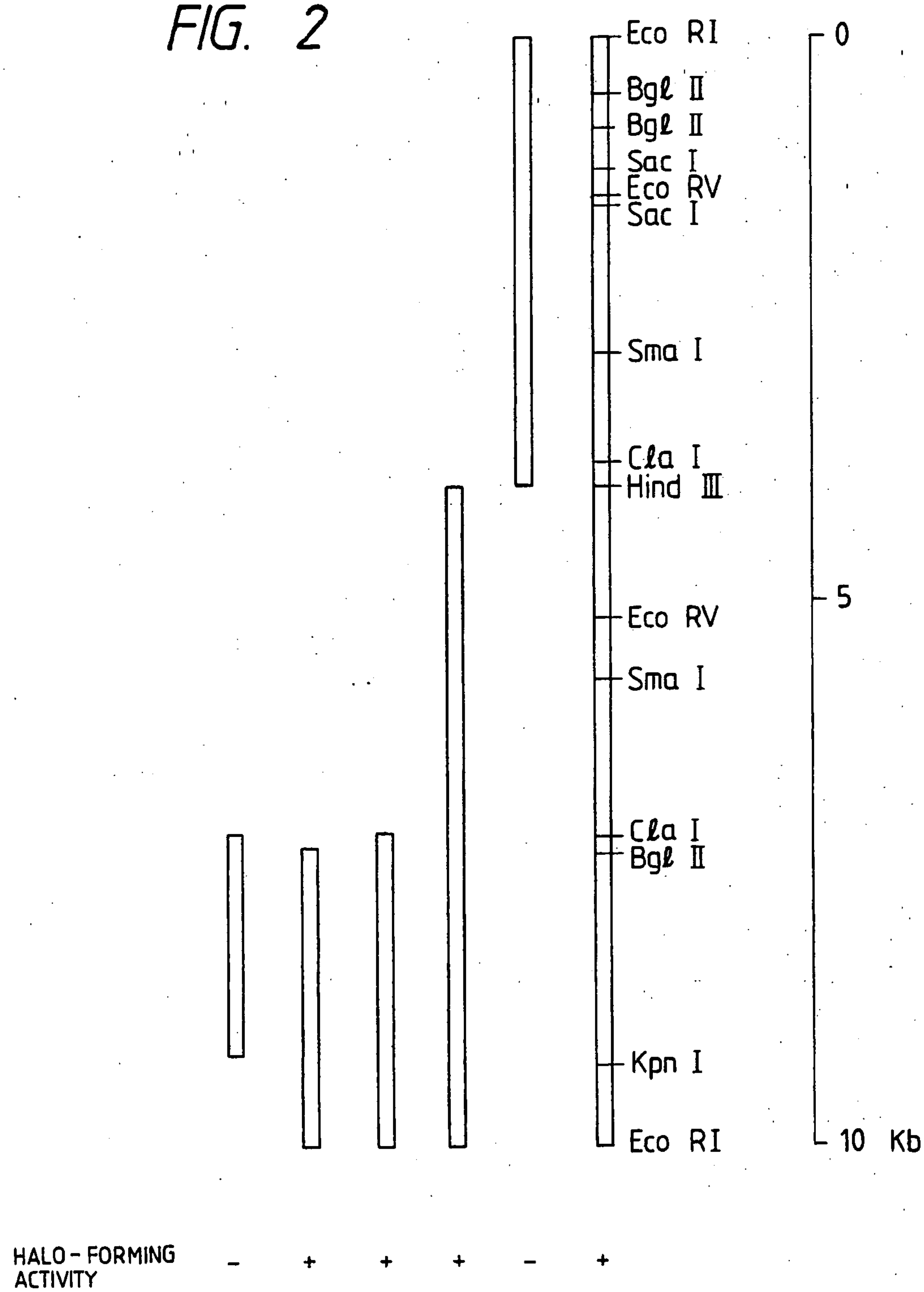
Eco RI
Bgl II
Bgl II
Sac I
Eco RV
Sac I
Sma I
Cla I
Hind III
Eco RV
Sma I
Cla I
Bgl II
Kpn I
Eco RI
0
5
10 Kb
HALO-FORMING ACTIVITY
-
+
+
+
-
+

FIG. 3A

```
                                      10                                      20
MetAlaArgSerMetArgSerArgValValAlaGlyAlaValAlaCysAlaMetSerVal

                                      30                                      40
AlaProPheAlaGlyMetThrAlaAlaMetThrLeuAlaThrThrArgAlaAlaMetAla

                                      50                                      60
AlaSerAlaProAlaAspAsnTyrAlaAlaThrArgTyrProIleIleLeuValHisGly

                                      70                                      80
LeuThrGlyThrAspLysTyrAlaGlyValLeuGluTyrTrpTyrGlyIleGlnGluAsp

                                      90                                     100
LeuGlnGlnArgGlyAlaTheValTyrValAlaAsnLeuSerGlyPheGlnSerAspAsp

                                     110                                     120
GlyProAsnGlyArgGlyGluGlnLeuLeuAlaTyrValLysThrValLeuAlaAlaThr

                                     130                                     140
GlyAlaThrLysValAsnLeuValGlyHisSerGlnGlyGlyLeuThrSerArgTyrVal

                                     150                                     160
AlaAlaValAlaProAspLeuValAlaSerValThrThrIleGlyThrProHisArgGly

                                     170                                     180
SerGluPheAlaAspPheValGlnGlyValLeuAlaTyrAspProThrGlyLeuSerSer

                                     190                                     200
ThrValIleAlaAlaPheValAsnValPheGlyIleLeuThrSerSerSerAsnAsnThr

                                     210                                     220
AsnGlnAspAlaLeuAlaAlaLeuLysThrLeuThrThrAlaGlnAlaAlaThrTyrAsn

                                     230                                     240
GlnAsnTyrProSerAlaGlyLeuGlyAlaProGlySerCysGlnThrGlyAlaProThr

                                     250                                     260
GluThrValGlyGlyAsnThrHisLeuLeuTyrSerTrpAlaGlyThrAlaIleGlnPro

                                     270                                     280
ThrIleSerValPheGlyValThrGlyAlaThrAspThrSerThrIleProLeuValAsp

                                     290                                     300
ProAlaAsnAlaLeuAspProSerThrLeuAlaLeuPheGlyThrGlyThrValMetVal

                                     310                                     320
AsnArgGlySerGlyGlnAsnAspGlyValValSerLysCysSerAlaLeuTyrGlyGln

                                     330                                     340
ValLeuSerThrSerTyrLysTrpAsnHisLeuAspGluIleAsnGlnLeuLeuGlyVal

                                     350                                     360
ArgGlyAlaAsnAlaGluAspProValAlaValIleArgThrHisAlaAsnArgLeuLys

LeuAlaGlyVal
```

FIG. 3B

```
                                    10                                      20
MetAlaSerArgAspGlyHisGlyArgArgValAlaGlyArgGlySerAlaGlyGlyAla

                                    30                                      40
AlaAlaAlaProProGlnAlaAlaLeuProAlaSerThrGlyLeuProSerSerLeuAla

                                    50                                      60
GlySerSerAlaProArgLeuProLeuAspAlaGlyGlyHisLeuAlaLysSerArgAla

                                    70                                      80
ValArgAspPhePheAspTyrCysLeuThrAlaGlnSerAspLeuSerAlaAlaAlaLeu

                                    90                                     100
AspAlaPheValValArgGlnIleAlaAlaGlnLeuAspGlyThrValAlaGlnAlaGlu

                                   110                                     120
AlaLeuAspValTrpHisArgTyrArgAlaTyrLeuAspAlaLeuAlaLysLeuArgAsp

                                   130                                     140
AlaGlyAlaValAspLysSerAspLeuGlyAlaLeuGlnLeuAlaLeuAspGlnArgAla

                                   150                                     160
SerIleAlaTyrArgThrLeuGlyAspTrpSerGlnProPhePheGlyAlaGluGlnTrp

                                   170                                     180
ArgGlnArgTyrAspLeuAlaArgLeuLysIleAlaGlnAspArgThrLeuThrAspAla

                                   190                                     200
GlnLysAlaGlnArgLeuAlaAlaLeuGluGlnGlnMetProAlaAspGluArgAlaAla

                                   210                                     220
GlnGlnArgValAspGlnGlnArgAlaAlaIleAspArgIleAlaGlnLeuGlnLysSer

                                   230                                     240
GlyAlaThrProAspAlaMetArgAlaGlnLeuThrGlnThrLeuGlyProGluAlaAla

                                   250                                     260
AlaArgValAlaGlnMetGlnGlnAspAspAlaSerTrpGlnSerAlaThrArgThrMet

                                   270                                     280
ArgArgSerValArgArgSerSerArgProAlaCysArgArgArgIleAlaThrProArg

                                   290                                     300
SerProHisCysGlySerAlaArgSerArgAsnProAlaLysArgCysGlyArgHisArg

                                   310
SerIleAlaAlaArgGlySerAlaAlaValThrArgAlaAlaArgCysAla
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	JOURNAL OF GENERAL MICROBIOLOGY, vol. 134, 1988, pages 433-440; S. WOHLFARTH et al.: "Chromosomal mapping and cloning of the lipase gene of Pseudomonas aeruginosa" * The whole document * ---	1-9,11	C 12 N 15/00 C 12 N 1/20 C 12 N 9/20 // (C 12 N 1/20 C 12 R 1:38)
Y	EP-A-0 204 284 (SAPPORO) * The whole document * ---	1-9,11	
Y	CURR. TOP. MICROBIOL. IMMUNOL. vol. 96, 1982, pages 47-67; M. BAGDASARIAN et al.: "Host: vector systems for gene cloning in pseudomonas" * The whole article * ---	1-9,11	
D,X	CHEMICAL ABSTRACTS, vol. 106, no. 11, 16th March 1987, page 143, abstract no. 79447q, Columbus, Ohio, US; W. KUGIMIYA et al.: "Molecular cloning and nucleotide sequence of the lipase gene from Pseudomonas fragi", & BIOCHEM. BIOPHYS. RES. COMMUN. 1986, 141(1), 185-90 * The whole abstract *	10	**TECHNICAL FIELDS SEARCHED (Int. Cl.4)**
			C 12 N
D,Y	IDEM -----	1-9,11	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	19-02-1990	PULAZZINI A.F.R.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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